

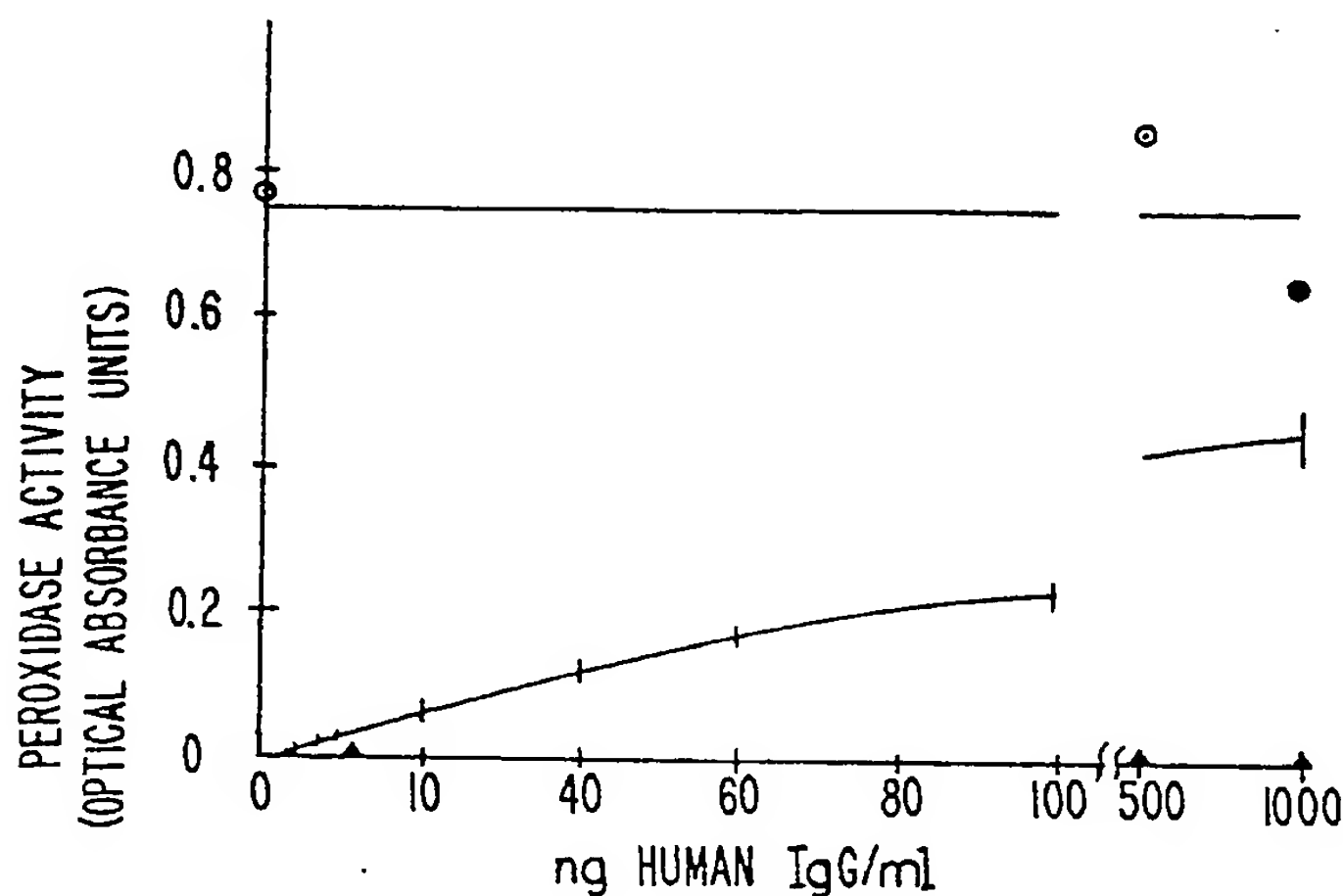
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: SOLID PHASE IMMUNOASSAY USING IMMUNOREAGENTS IMMOBILIZED ON INERT SYNTHETIC RESIN SURFACES



## (57) Abstract

A solid phase immunoassay comprises the steps of a) immobilizing an immunoreagent on the surface of a carrier comprised of an inert synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins, b) contacting the immunoreagent with a complementary immunoreagent whereby an immunocomplex immobilized on said carrier is formed, c) quantitating the immobilized immunocomplex. An element useful in conducting this solid phase immunoassay is prepared by a process of treating the surface of an article comprised of a synthetic polymer selected from the group consisting of polyimides and polyfluorinated synthetic resins to make it adsorptive of an immunoreagent which comprises the steps of a) thoroughly rinsing the surface with a water-miscible organic solvent, b) thoroughly rinsing the surface with water.

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5     TITLE:           SOLID PHASE IMMUNOASSAY USING IMMUNO-  
                          REAGENTS IMMOBILIZED ON INERT SYNTHETIC  
                          RESIN SURFACES

BACKGROUND OF THE INVENTION

10

Field of the Invention:

          This invention relates to solid phase immunoassays  
          using immobilized immunoreagents and more particularly  
          to elements for solid phase immunoassay comprising  
15   immunoreagents immobilized on carriers made of inert  
          synthetic resins. The invention also relates to meth-  
          ods of preparing synthetic resin surfaces for use as  
          carriers for immunoreagents.

20

Description of the Prior Art:

          Immunoassay using immobilized immunoreagents is an  
          analytical method widely used in biochemical analysis.  
          In the conventional procedure an immunoreagent, e. g.,  
          an antigen or antibody, is first immobilized on the  
25   surface of an analytical element, e. g., a test tube, a  
          rod or stick, beads of glass or plastic, or the like.  
          The immobilized immunoreagent is then contacted with an  
          analyte solution containing a complementary immunorea-  
          gent, whereby an immobilized immunocomplex is formed.  
30   The immobilized immunocomplex can then be easily sepa-  
          rated from the unreacted analyte solution, e. g., by  
          simply removing the analyte solution by aspiration,  
          decantation, or the like, preferably with repeated  
          washing of the immobilized immunocomplex. The separa-  
35   ted immobilized immunocomplex may then be subjected to  
          further processing to quantitate the amount of immuno-  
          complex. For example, in a radioimmunoassay, the

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5 amount of adsorbed complex may be determined by counting radioactive disintegrations; in an enzyme-linked immunosorbent assay (ELISA) the adsorbed complex which has an enzyme coupled thereto, is contacted with a substrate for the enzyme to produce a detectable product; in an immunofluorescent assay, the fluorescent intensity of a fluorescent substance linked to the immunocomplex may be measured, or the like.

10 Among the many different materials which have been used as carriers for an immunoreagent are glass, metal, and various plastics such as polystyrene, polyvinyl chloride, silicone resins, polyethylene and the like. In some cases the immunoreagent has been immobilized by covalent bonding to the carrier, while in other cases adsorption of the immunoreagent has proved adequate. 15 Synthetic resin carriers have been widely used because of their economy and convenience and ease of handling, but problems remain in immobilizing enough immunoreagent on their surfaces for maximum sensitivity of the immunoassay, especially when the reagent is merely adsorbed onto the surface. Furthermore, the synthetic resin carriers used previously are inadequate for some of the newer immunoassay techniques such as thermochemiluminescent immunoassay, which requires that the carrier with immobilized immunocomplex be heated to relatively high temperatures, i. e., 200°C to 300°C. The conventional plastic carriers cannot be used under these conditions because they soften or even melt at such temperatures. 25 30

35 On the other hand, certain plastics are known which can be used at temperatures of 200° to 300°C without melting or deformation, for example, polyimide synthetic resins, and polytetrafluoroethylene (PTFE) and related fluorinated olefin polymers. However, these resins are very inert and non-adhesive, and it

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5 has been thought that satisfactory adsorption of immunoreagents to such surfaces was not possible.

Prior workers in this field do not appear to have attempted to use a polyimide as a carrier for an immunoreagent in a solid phase immunoassay.

10 Some attempts have been made to use PTFE as a carrier for solid phase immunoassay, but the procedures have been unsuccessful or have had serious drawbacks.

Shekarchi, et al., J. Clin. Microbiology 16(6), 1012-1018 (Dec., 1982) disclose an immunoassay procedure wherein an immunoreagent is immobilized on a small stick, i. e., "microstick", for easy manipulation of the reagent and the immunocomplex. While a number of materials were investigated for use in such microsticks, including stainless steel, nylon, polycarbonate, polystyrene and PTFE, it was found that the PTFE, cleaned by the conventional procedure of rinsing with 6 N HCl, adsorbed very little of the immunoreagent as compared with the other materials and could not be used as a base for the immunoreagent until it had been coated with polycarbonate or nitrocellulose.

25 German Offenlegungsschrift 32 00 822, published July 21, 1983, discloses a method for activating the surface of PTFE articles, in order to bond immunoreagents covalently, by contacting the PTFE surface with an ammoniacal solution of sodium, followed by treatment with carbodiimide. The process was apparently attempted because it was found that adsorption of the immunoreagent on PTFE was unsatisfactory. This process is complex and uses reagents which are difficult to handle and even dangerous. Furthermore, there is some question whether the procedure of this German application actually can immobilize a useful amount of immunoreagent on PTFE.

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5           Hence, a need has continued to exist for improved  
immunoassay procedures using immunoreagents immobilized  
on inert carriers such as polyimides and fluorinated  
polymers and for a practical method of immobilizing  
10           immunoreagents on the surfaces of such synthetic res-  
ins.

#### SUMMARY OF THE INVENTION

          It has now been found that a solid phase immunoas-  
say element comprising an immunoreagent adsorbed onto  
15           the surface of a heat resistant synthetic resin such as  
a polyimide or PTFE can be prepared by adsorbing the  
immunoreagent onto the surface by simply incubating the  
surface in an aqueous solution of the immunoreagent,  
provided that the surface of the synthetic resin has  
20           first been cleaned by thorough rinsing with a water-  
miscible organic solvent followed by thorough rinsing  
with water.

          The invention further comprises a solid phase  
immunoassay procedure using an immunoreagent immobil-  
25           ized on a carrier made of a polyimide or a polyfluori-  
nated synthetic resin.

          The invention further comprises a process for  
treating the surface of an article comprised of a syn-  
thetic polymer selected from the group consisting of  
30           polyimides and polyfluorinated synthetic resin polymers  
to make it receptive to adsorption of an immunoreagent  
this process comprising the steps of

          a) thoroughly rinsing the surface with a water-  
miscible organic solvent, and

35           b) thoroughly rinsing the surface with water.

          An immunoreagent may then be adsorbed onto the  
surface of the cleaned synthetic resin by contacting  
the surface with an aqueous solution of the immunorea-  
gent.

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5           Thus, it is an object of the invention to provide a method of solid phase immunoassay using an immunoreagent immobilized on the surface of an inert synthetic resin.

10           A further object is to provide an immobilized immunoreagent.

          A further object is to provide an immunoreagent immobilized on a synthetic resin surface, where the synthetic resin is a polyimide or a polyfluorinated synthetic resin.

15           A further object is to provide a method for treating an inert synthetic resin surface to make it adsorptive to immunoreagents.

          A further object is to provide a method of preparing an immobilized immunological reagent.

20           A further object is to provide a method of immobilizing an immunological reagent on a polyimide synthetic resin surface.

          A further object is to provide a method of immobilizing an immunological reagent on a polyfluorinated polymer surface.

25           Further objects of the invention will become apparent from the description of the invention which follows.

30           BRIEF DESCRIPTION OF THE DRAWINGS

          Figure 1 illustrates the results of the solid phase enzyme-linked immunosorbent assay of Example 3 using a conventional polystyrene carrier.

35           Figure 2 illustrates the results of the solid phase enzyme-linked immunosorbent assay of Example 3 using a polyimide resin carrier.

          Figure 3 illustrates the results of the solid phase enzyme-linked immunosorbent assay of Example 3 using a PTFE carrier.



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DETAILED DESCRIPTION OF THE INVENTION  
AND PREFERRED EMBODIMENTS

10

The solid phase immunoassay of the invention using an immunoreagent immobilized on an inert resin surface of a polyimide or a polyfluorinated synthetic resin is capable of giving greater sensitivity than assays using immunoreagents immobilized on conventional carriers such as polystyrene and the like.

15

Solid phase immunoassay elements comprising an immunoreagent immobilized on a carrier surface of a polyimide or polyfluorinated synthetic resin are essential to the practice of the immunoassay of the invention. Such elements are useful in the practice of conventional immunoassays, but are especially useful for thermochemiluminescence immunoassays which require that the carrier with an immobilized immunocomplex be heated to relatively high temperatures, e. g., 200°C to 300°C.

20

The solid phase immunoassay elements of this invention use carriers comprised of a polyimide or a polyfluorinated synthetic resin. The polyimide resins are inert heat-resistant synthetic resins which are characterized by the presence of the phthalimide structure in the backbone. A preferred polyimide is a condensation polymer of pyromellitic acid and bis(4-aminophenyl)oxide, sold by E. I. du Pont de Nemours & Co. under the name Kapton. The process may also employ polyfluorinated synthetic resins having surfaces of low surface energy which have hitherto been thought to be too non-adhesive for adsorption of immunoreagents. Such polymers include polymers of perfluorinated carbonates, polymers of perfluorinated epoxy compounds and especially addition polymers of ethylenically unsaturated hydrocarbons such as polytetrafluoroethylene, poly(chlorotrifluoroethylene) and

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30

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5 the like. These materials are manufactured by a number of suppliers, e. g., by the Du Pont Co., under the name Teflon .

10 The water-miscible organic solvent used in the preparation of the immunoassay elements of the invention may be any solvent which can produce the requisite cleanliness of the surface of the synthetic resin, and is sufficiently miscible with water to be completely removed by subsequent thorough rinsing with water. Such solvents include lower aliphatic alcohols, e. g.,  
15 C<sub>1</sub>-C<sub>4</sub> aliphatic alcohols such as methanol, ethanol, n-propanol, isopropyl alcohol, n-butanol, isobutanol, sec.-butyl alcohol and tert.-butyl alcohol; lower aliphatic ketones, e. g., acetone, methyl ethyl ketone, and the like, dioxan, dimethylformamide, dimethyl sulf-  
20 oxide, acetonitrile, lower (C<sub>1</sub>-C<sub>4</sub>) glycols such as ethylene glycol and propylene glycol, and lower aliphatic ethers having a total of about 3 to 6 carbon atoms, e. g., 2-methoxyethanol, 2-ethoxyethanol and the like. Mixtures of solvents may also be used. Preferred sol-  
25 vents are lower aliphatic alcohols, and a most preferred solvent is ethanol.

The solvent rinsing step is preferably conducted by contacting the surface of the synthetic resin with clean solvent for a period of time required to thor-  
30 oughly clean the surface. The exact experimental conditions may vary with the solvent chosen, but the evaluation of the results in order to choose the proper condition may be simply carried out by using the surface in a standard immunoassay. A surface which has  
35 been inadequately cleaned will poorly adsorb the immunoreagent and manifest this poor adsorption by a low sensitivity in the immunoassay. It is preferable to have the solvent which contacts the synthetic resin surface as free as possible of contaminants. This can

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5 be accomplished by continuously supplying fresh solvent  
to the surface, e. g., in a flowing system, or by fre-  
quent changes of solvent when the cleaning is conducted  
by a batch process. The solvent will ordinarily be  
10 used in an anhydrous state for maximum solvent power  
for organic contaminants on the resin surface. How-  
ever, the invention is also intended to include the use  
of solvents containing small amounts of water which do  
not seriously interfere with the solvent properties of  
the solvents toward organic surface contaminants.

15 It is preferred to conduct the solvent rinsing  
step by contacting the surface with the solvent at its  
boiling temperature. Accordingly water-miscible sol-  
vents having boiling points between about 40°C and  
about 200°C are preferred. The solvent rinsing step is  
20 therefore conveniently carried out by placing the arti-  
cles to be coated, e. g., disks of the polymer to be  
used in immunosorbent assays, in a flask containing the  
solvent and heating to reflux temperature. The time of  
the solvent rinsing step will also vary depending on  
25 the solvent and its temperature. A relatively short  
contact time, e. g., one-half hour, may produce a sur-  
face which will adsorb some immunoreagent. However, it  
is preferable to keep the surface in contact with the  
solvent for a relatively long period of time, e. g.,  
30 one or two days, in order to assure maximum cleaning  
and maximum adsorption of immunoreagent, which assures  
the highest sensitivity of the immunoassay. It is also  
preferable to change the solvent several times in the  
course of the rinsing step to insure maximum cleanli-  
35 ness of the solvent in the final portion of the rinsing  
step.

A preferred procedure is to contact the surface of  
the synthetic resin carrier with ethanol at refluxing

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5 temperature for a period of one to two days with frequent changes of solvent.

After the completion of the solvent cleaning step, the solvent-rinsed surface is then thoroughly rinsed with pure water, e. g., distilled or deionized water, to remove all traces of the solvent. Again, the preferable procedure is to immerse the articles in boiling water in a refluxing apparatus, for several hours, with several changes of water. The presence of residual solvent on the surface of the synthetic resin interferes with the adsorption of the immunoreagent and hence insufficient water rinsing is readily detected by the practitioner by simple use of a standard immunoassay. Evidently, the necessary length of the water rinsing step and the number of changes of fresh water can be easily determined by the practitioner using this criterion.

If desired the carriers may be dried after the water rinsing step. The drying is preferably conducted by draining the water from the carriers and allowing the residual water to evaporate. It is preferable to assure complete dryness, especially when the carriers are to be used for a thermoluminescent assay, by drying in an oven at an elevated temperature, e. g., 200°C to 300°C, for an extended period of time. Preferred drying conditions are 300°C overnight.

The articles prepared by the cleaning process, either immediately after the rinsing step, without drying the surface, or after the surface has been dried, may then be coated with an immunoreagent by a relatively conventional adsorption step wherein the articles are contacted with an aqueous solution of the immunoreagent in a suitable buffer for a period of time to allow physical adsorption to occur to a sufficient extent. The basic process for this adsorption step is

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5 disclosed by Catt, U. S. Patent 3,646,346. For exam-  
ple, the article may be contacted with an aqueous solu-  
tion of an antibody at a concentration of 0.1 mg/ml in  
0.1 M tris(hydroxymethyl)aminomethane (Tris) at a pH of  
10 about 7.6 at 4°C for an extended period of time, e. g.  
2 or more days. The coated articles are then thor-  
oughly washed with distilled water and are ready for  
use in immunoassay procedures.

Any immunoreagent conventionally used in immuno-  
assay can be adsorbed to the surface of the inert  
15 synthetic resin carrier used in this invention.ordi-  
narily, the immunoreagent will be an antibody or an  
antigen and generally the immunoreagent will be a pro-  
teinaceous material.

While the preferred conditions are given above,  
20 effective ranges are rather broad, and it will be  
understood by those skilled in the art that the  
conditions may be varied, provided that the essential  
steps are performed. The success of the treatment  
under a given set of conditions is easily evaluated by  
25 simply measuring the amount of immunoreagent absorbed,  
e. g., by use in a conventional standard immunoassay.

The invention will be illustrated by the following  
examples which are not intended to be limiting. In the  
examples all parts and percentages are by weight.

30 Example 1

This example illustrates the surface treatment  
process of process of this invention and immobilization  
of immunoreagent.

About 150 disks of a polyimide synthetic resin  
35 (Kapton 500H, manufactured by the du Pont company)  
having a diameter of 9 mm and a thickness of 125 micro-  
meters were placed in a flask equipped with a reflux  
condenser, covered with ethanol, and rinsed with etha-  
nol at reflux temperature for a period of one day with

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5 several changes of the ethanol solvent. The disks were then rinsed with distilled water at reflux temperature for several hours with several changes of distilled water. The disks were then removed from the flask and dried in an oven at a temperature of 300°C overnight.

10 The dried disks were then placed in 40 ml of a solution of 0.1 mg/ml of antibody in 0.1 M Tris buffer (pH 7.6) and 0.02 %  $\text{NaN}_3$ , at a temperature of 4°C. The disks were gently shaken during the coating procedure which was continued for a period of 2 days. The coated

15 disks were then washed in distilled water and could be preincubated, if desired, to prepare them for use in immunoassay by incubating them in an aqueous solution of 4 % bovine serum albumin (BSA) in a 10 mM Tris buffer (pH 8.0), 0.15 M NaCl, 0.05 % polyoxyethylene sor-

20 bitan surfactant (Tween 20 ) (Buffer A) for at least one hour.

#### Example 2

The procedure of Example 1 was used to prepare and coat about 150 disks of polytetrafluoroethylene having

25 a diameter of 9 mm and a thickness of 500 micrometers.

#### Example 3

This example illustrates a comparison of ELISA conducted with the immunological reagent adsorbed by conventional procedures on a polystyrene microtiter

30 plate and on carriers coated according to this invention.

Disks of polyimide or of polytetrafluorethylene were coated by the procedure of Example 1 with goat antihuman Immunoglobulin G (IgG) (7S) (Nordic), human

35 IgG (Sigma) and BSA (Sigma). Polystyrene microtiter plates (Costar, Holland) were coated for one week with the same antibodies and with BSA by contacting them for a period of one week with a solution of 0.1 mg protein per ml, 0.1 M carbonate buffer, pH 9.6, at a tempera-

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5        ture of 4°C. Each well of the plates contained 0.1 ml of the solution. After the different carrier polymers were coated with the different immunoreagents, the solid phase immunoassay procedures were identical.

10        The coated carriers were preincubated for one hour with 0.2 ml of Buffer A. After washing with distilled water, the disks were incubated with 0.2 ml human IgG standards (0; 1; 2.5; 5; 7.5; 10; 20; 40; 60; 100; 500; 1000 ng human IgG per ml of Buffer A) for one hour at 37°C while shaking in a waterbath. Subsequently, the  
15        disks were again washed with distilled water by decantation and incubated with 0.2 ml of an aqueous solution containing 1 microgram of peroxidase labeled goat-antihuman conjugate (Nordic) per milliliter of Buffer A at 37°C in a waterbath with shaking. After removal of  
20        unbound conjugate, peroxidase activity on the disks was measured by means of the conversion of ortho-phenylenediamine to a colored product. Carriers coated with immunoreagent were incubated in the dark with 0.2 ml of M-phosphate buffer, pH 5.0, 0.0045 %  $H_2O_2$ ,  $2 \cdot 10^{-3}$  M  
25        ortho-phenylenediamine HCl (UCB) for one hour. Color development was stopped by adding of 0.5 ml of 1 N  $H_2SO_4$ . The optical density of the color was measured at 495 nm with a Zeiss spectrophotometer.

30        Figure 1 shows the results of an immunoassay on a polystyrene carrier. Figure 2 shows the results of the same immunoassay using the polyimide (Kapton 500H ) disks coated by the process of this invention. Figure 3 shows the results of the same immunoassay performed using PTFE disks coated by the process of this invention.  
35        tion.

      In the figures the symbols I indicate the values measured for the series of human IgG standards. Each data bar represents a triplicate measurement. The vertical length of the bar represents the range of the



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5 repeated determinations. Note that at a concentration  
below 20 ng/ml the measurements are so reproducible  
that the data bar has hardly any vertical length. The  
triangles indicate the measured values for the negative  
control (BSA) and the circles represent the measured  
10 values for the positive control (disks coated with pure  
antigen, human IgG).

Figures 2 and 3 show that the peroxidase activity  
of the adsorbed immunocomplex is substantially greater  
for the polyimide and PTFE carriers prepared according  
15 to this invention than for the conventional polystyrene  
carrier. Accordingly, is evident from the results of  
the experiment that the inert synthetic resin surfaces  
prepared and coated with immunoreagent by the process  
of this invention yield results superior to the conven-  
20 tional immunoassay using polystyrene microtiter plates.

#### Example 4

This example illustrates the results of compara-  
tive immunoassays using polyimide carriers prepared by  
cleaning processes different from that of this inven-  
25 tion.

##### A. Acidic cleaning treatment.

Kapton disks were contacted with 98 % sulfuric  
acid for one minute at room temperature, washed with  
distilled water, and immediately (without drying) incu-  
30 bated with either human serum albumin (antigen) or  
human IgG (non-antigen). The incubated disks were then  
contacted with an aqueous solution of peroxidase-  
labeled rabbit-anti-human serum albumin (antibody). It  
was found that the amount of antibody bound to the  
35 carrier was the same for both the antigen-coated car-  
rier and the carrier coated with non-antigen protein.  
Accordingly the antibody binding of the polyimide car-  
rier prepared by an acid cleaning treatment is com-  
pletely non-specific, and such a carrier is useless for



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5 solid phase immunoassay.

B. Basic cleaning treatment

Polyimide disks were treated with 5 % KOH in dis-  
tilled water for 5 minutes at room temperature, then  
washed a few times with distilled water. Analytical  
10 disks were then incubated with a series of human IgG  
standards of various concentrations according to the  
procedure of Example 3, while control disks were coated  
by incubation with a solution of bovine serum albumin  
(BSA). The immunoassay was conducted by the procedure  
15 of Example 3. It was found that the blank value of  
bound peroxidase activity (i. e., for the disks coated  
with BSA) was very high, about 0.80 to about 1.0 opti-  
cal absorbance units. While the measured values of  
bound peroxidase activity for the disks coated with  
20 human IgG were measurably greater than the blank value,  
it is evident that such a high blank value produces an  
assay having inferior sensitivity and accuracy.

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Example 5

5        This example illustrates the specific nature of  
the pretreatment process of this invention

10        Glass disks about 1.0 cm in diameter were treated  
by the process of Example 1 and used in an immunoassay  
as described in Example 3. It was found that the blank  
value (BSA coated disks) was rather high, about 0.25  
optical absorbance units, while the measured value for  
an analyte concentration of 100 ng/ml (human IgG) was  
only about 0.40, i. e., only about 0.15 optical absorb-  
15        ance units greater than the blank value. The same  
process applied to glass beads about 5-7 mm in diameter  
gave even poorer results; the beads appeared to have no  
specific binding power after being subjected to the  
conventional adsorption process to coat them with an  
immunoreagent. Such results indicate that this process  
20        is not suitable for use with glass carriers. Accord-  
ingly, it is evident that the process of the invention  
is not a general cleaning process, but rather a clean-  
ing process specially adapted to cleaning and preparing  
carriers of the claimed synthetic resins.

25        The invention having now been fully described, it  
should be understood that it may be embodied in other  
specific forms or variations without departing from its  
spirit or essential characteristics. Accordingly, the  
embodiments described above are to be considered in all  
30        respects as illustrative and not restrictive, the scope  
of the invention being indicated by the appended claims  
rather than by the foregoing description, and all  
changes which come within the meaning and range of  
equivalency of the claims are intended to be embraced  
35        therein.

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## 5 WHAT IS CLAIMED IS:

1. A solid phase immunoassay comprising the steps of

10 a) immobilizing an immunoreagent on the surface of a carrier comprised of an inert synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins,

15 b) contacting said immunoreagent with a complementary immunoreagent whereby an immunocomplex immobilized on said carrier is formed,

c) quantitating said immobilized immunocomplex.

20 2. The immunoassay of Claim 1 wherein said synthetic resin is a polyimide.

3. The immunoassay of Claim 2 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

25 4. The immunoassay of Claim 1 wherein said synthetic resin is a polyfluorinated synthetic resin.

30 5. The immunoassay of Claim 4 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

6. The immunoassay of Claim 5 wherein said polyfluorinated synthetic resin is polytetrafluoroethylene.

35 7. The immunoassay of Claim 1 wherein said immunoreagent is a protein.

8. The immunoassay of Claim 1 wherein said immunoreagent is an antibody.

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5           9. The immunoassay of Claim 1 wherein said immunoreagent is an antigen.

10           10. An element for use in a solid phase immunoassay comprising an immunoreagent immobilized on the surface of a carrier comprised of an inert synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins.

15           11. The element of Claim 10 wherein said synthetic resin is a polyimide.

20           12. The element of Claim 11 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

            13. The element of Claim 10 wherein said synthetic resin is a polyfluorinated synthetic resin.

25           14. The element of Claim 13 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

            15. The element of Claim 14 wherein said polyfluorinated synthetic resin is polytetrafluoroethylene.

30           16. The element of Claim 10 wherein said immunoreagent is a protein.

            17. The element of Claim 10 wherein said immunoreagent is an antibody.

35

            18. The element of Claim 10 wherein said immunoreagent is an antigen.

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5           19. A process for treating the surface of an article comprised of a synthetic polymer selected from the group consisting of polyimides and polyfluorinated synthetic resins to make it receptive to adsorption of an immunoreagent, said process comprising the steps of

- 10           a) thoroughly rinsing the surface with a water-miscible organic solvent,  
            b) thoroughly rinsing the surface with water.

15           20. The process of Claim 19 wherein said synthetic resin is a polyimide.

            21. The process of Claim 20 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

20           22. The process of Claim 19 wherein said synthetic resin is a polyfluorinated synthetic resin.

25           23. The process of Claim 22 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

            24. The process of Claim 23 wherein said polyfluorinated synthetic resin is polytetrafluoroethylene.

30           25. The process of Claim 19 wherein said solvent is a C<sub>1</sub>-C<sub>4</sub> aliphatic alcohol.

            26. The process of Claim 25 wherein said alcohol is ethanol.

35           27. The process of Claim 19 wherein said solvent rinsing is carried out at a temperature equal to boiling point of the solvent.

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5           28. The process of Claim 19 wherein said solvent rinsing step is carried out for a period of one-half hour to four days.

10           29. The process of Claim 28 wherein said solvent rinsing step is carried out for at least one day.

15           30. The process of Claim 19 wherein said water rinsing is carried out at a temperature equal to the boiling point of water.

            31. The process of Claim 19 additionally comprising the step of

            b') drying said surface after step b).

20           32. The process of Claim 31 wherein said drying step is carried out at a temperature greater than 200°C.

            33. The process of Claim 32 wherein said drying step is carried out at a temperature of about 300°C.

25           34. A process for preparing an element suitable for use in a solid phase immunoassay, said process comprising the steps of

30           a) thoroughly rinsing a surface of an article comprised of a synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins with a water-miscible organic solvent,

            b) thoroughly rinsing the surface with water, and

35           c) contacting the surface with an aqueous solution of an immunoreagent.

            35. The process of Claim 34 wherein said synthetic resin is a polyimide.

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5           36. The process of Claim 35 wherein said poly-  
imide is a polymer of pyromellitic acid and  
bis(4-aminophenyl)oxide.

10           37. The process of Claim 34 wherein said syn-  
thetic resin is a polyfluorinated synthetic resin.

15           38. The process of Claim 37 wherein said poly-  
fluorinated synthetic resin is a polymer of a poly-  
fluorinated ethylenically unsaturated hydrocarbon.

          39. The process of Claim 38 wherein said poly-  
fluorinated synthetic resin is polytetrafluoroethylene.

20           40. The process of Claim 34 wherein said solvent  
is a C<sub>1</sub>-C<sub>4</sub> aliphatic alcohol.

          41. The process of Claim 40 wherein said alcohol  
is ethanol.

25           42. The process of Claim 34 wherein said solvent  
rinsing is carried out at a temperature equal to boil-  
ing point of the solvent.

30           43. The process of Claim 34 wherein said solvent  
rinsing step is carried out for a period of one-half  
hour to four days.

          44. The process of Claim 34 wherein said solvent  
rinsing step is carried out for at least one day.

35           45. The process of Claim 34 wherein said water  
rinsing is carried out at a temperature equal to the  
boiling point of water.



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5           46. The process of Claim 34 additionally comprising the step of

b') drying said surface after step b) and before step c).

10           47. The process of Claim 46 wherein said drying step is carried out at a temperature greater than 200°C.

15           48. The process of Claim 47 wherein said drying step is carried out at a temperature of about 300°C.

20           49. A carrier for an immunoreagent to be used in a solid phase immunoassay, said carrier comprising an article having a surface comprised of a synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins, said surface having been treated by a process comprising the steps of

a) thoroughly rinsing the surface with a water-miscible organic solvent, and

25           b) thoroughly rinsing the surface with water.

50. The carrier of Claim 49 wherein said synthetic resin is a polyimide.

30           51. The carrier of Claim 50 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

35           52. The carrier of Claim 49 wherein said synthetic resin is a polyfluorinated synthetic resin.

53. The carrier of Claim 52 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

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5           54. The carrier of Claim 53 wherein said poly-  
fluorinated synthetic resin is polytetrafluoroethylene.

55. The carrier of Claim 49 wherein said solvent  
is a C<sub>1</sub>-C<sub>4</sub> aliphatic alcohol.

10

56. The carrier of Claim 55 wherein said alcohol  
is ethanol.

15           57. The carrier of Claim 49 wherein said solvent  
rinsing is carried out at a temperature equal to boil-  
ing point of the solvent.

20           58. The carrier of Claim 49 wherein said solvent  
rinsing step is carried out for a period of one-half  
hour to four days.

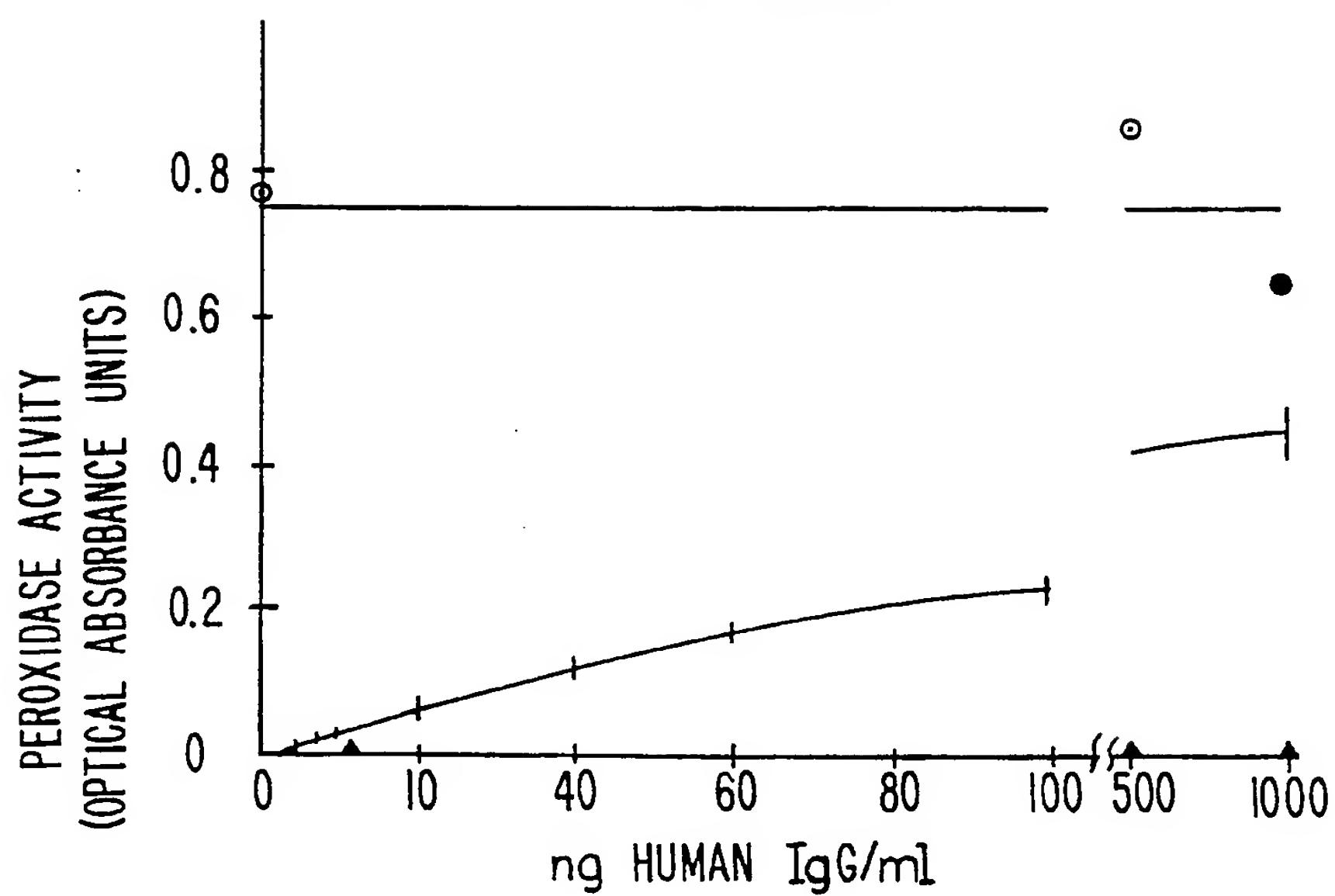
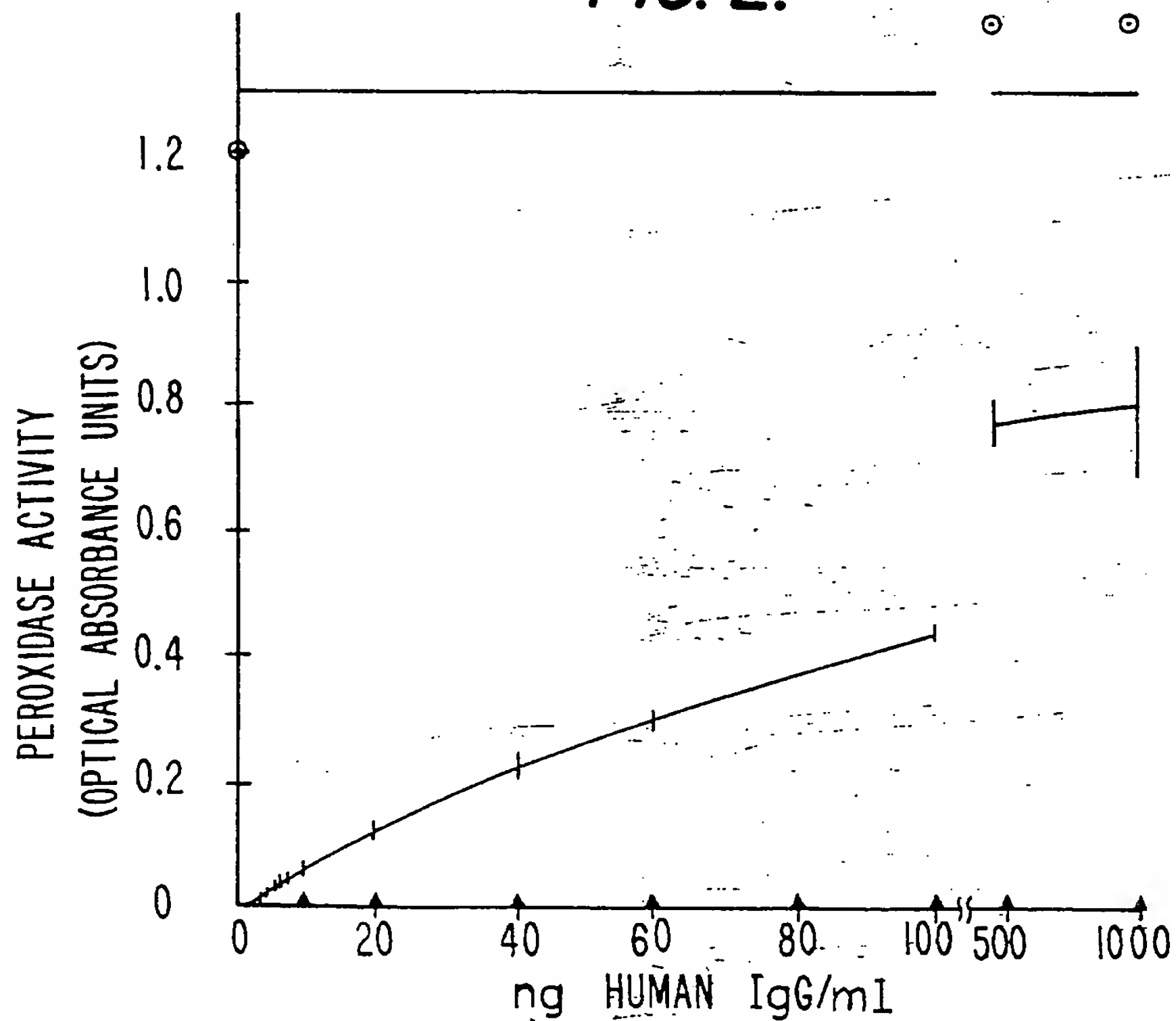
59. The carrier of Claim 49 wherein said solvent  
rinsing step is carried out for at least one day.

25           60. The carrier of Claim 49 wherein said water  
rinsing is carried out at a temperature equal to the  
boiling point of water.

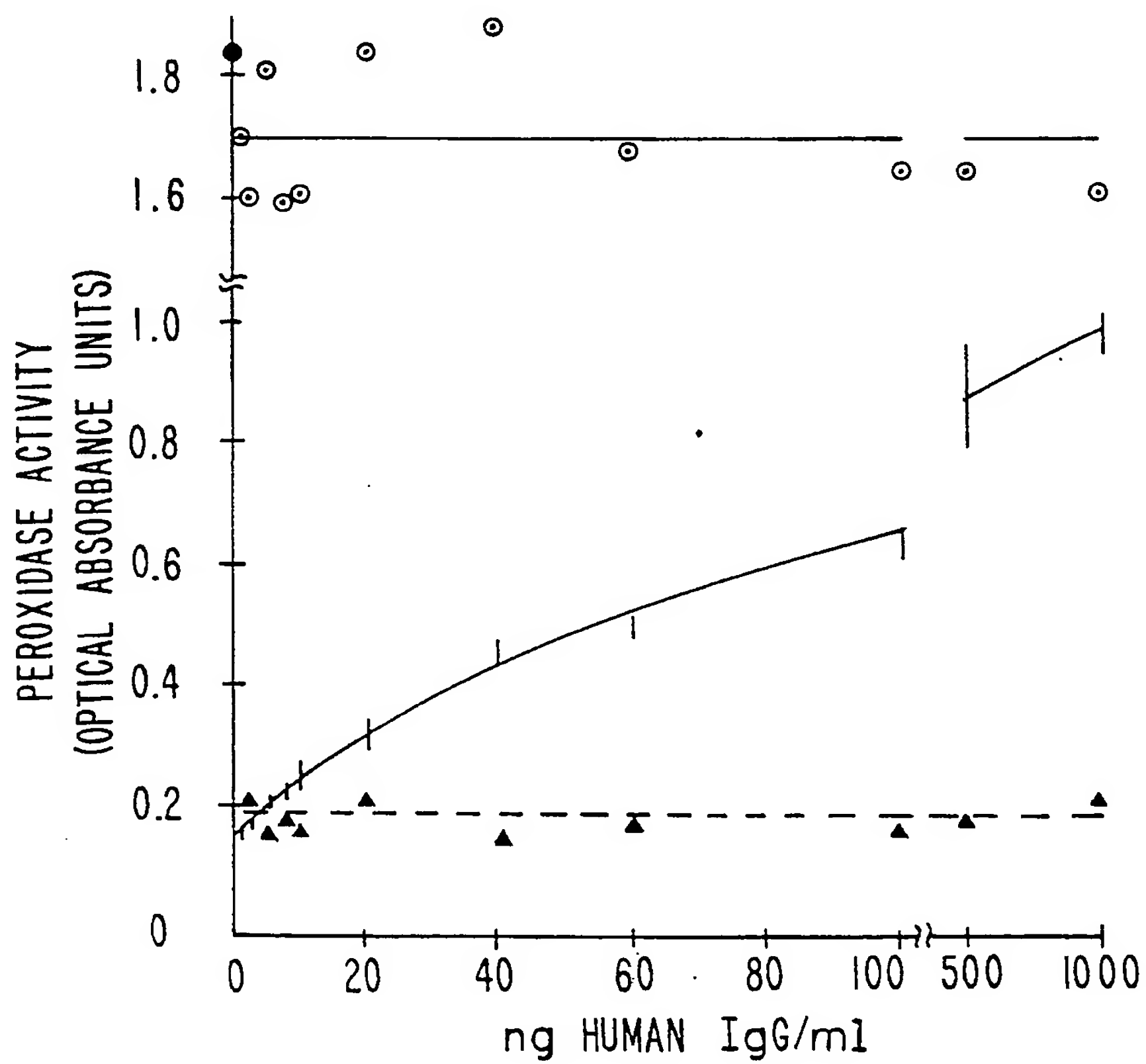
30           61. The carrier of Claim 49 additionally compris-  
ing the step of  
b') drying said surface after step b).

35           62. The carrier of Claim 61 wherein said drying  
step is carried out at a temperature greater than  
200°C.

63. The carrier of Claim 62 wherein said drying  
step is carried out at a temperature of about 300°C.

1/2 **FIG. 1.****FIG. 2.**

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**FIG. 3.**

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/02504

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>3</sup> According to International Patent Classification (IPC) or to both National Classification and IPC US Cl. 436-531, 534, 823, 824; 435-7 Int Cl. IV- G 01 N 33/545, 546, 53																				
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched <sup>4</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">US Cl.</td> <td style="padding: 5px;">436-531, 534, 823, 824 435-7</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>5</sup></div> <p style="padding: 5px;">Dialog 2 files 399,5,55,154,76,73,72,350,351 Lexpat (Mead Data)</p>			Classification System	Classification Symbols	US Cl.	436-531, 534, 823, 824 435-7														
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<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border-bottom: 1px solid black;">Category <sup>*</sup></th> <th style="width: 60%; border-bottom: 1px solid black;">Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup></th> <th style="width: 30%; border-bottom: 1px solid black;">Relevant to Claim No. <sup>18</sup></th> </tr> <tr> <td style="vertical-align: top; padding: 5px;">X,Y</td> <td style="padding: 5px;">US, N, "Coupling of proteins and haptens on Teflon for immunological assay", Chemical abstracts, Vol. 99 issued September 1983, abstract number 101950j, Von Klitzing et al (entire abstract).</td> <td style="vertical-align: top; padding: 5px;">1,4-10, 13-18,49, 52-54,61</td> </tr> <tr> <td style="vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">US, A, 4,017,597, published 12 April 1977, Reynolds, see column 10, lines 14-20.</td> <td style="vertical-align: top; padding: 5px;">1,4-10,13- 18,49,52-54, 61</td> </tr> <tr> <td style="vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">US, A, 4,444,879, published 24 April 1984, Foster et al, see column 3, lines 32-37</td> <td style="vertical-align: top; padding: 5px;">1,4-10;13- 18,49,52-54, 61</td> </tr> <tr> <td style="vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">US, A, 4,360,358, published 23 November 1982 Sharma, see column 5, line 58- column 6, line 17</td> <td style="vertical-align: top; padding: 5px;">1,4-10,13- 18,49,52-54, 61</td> </tr> <tr> <td style="vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">US, A, 4,357,142, published 2 November 1982, Schall, Jr. et al, see column 6, lines 37-64</td> <td style="vertical-align: top; padding: 5px;">19,34</td> </tr> </table>			Category <sup>*</sup>	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>	X,Y	US, N, "Coupling of proteins and haptens on Teflon for immunological assay", Chemical abstracts, Vol. 99 issued September 1983, abstract number 101950j, Von Klitzing et al (entire abstract).	1,4-10, 13-18,49, 52-54,61	X	US, A, 4,017,597, published 12 April 1977, Reynolds, see column 10, lines 14-20.	1,4-10,13- 18,49,52-54, 61	X	US, A, 4,444,879, published 24 April 1984, Foster et al, see column 3, lines 32-37	1,4-10;13- 18,49,52-54, 61	X	US, A, 4,360,358, published 23 November 1982 Sharma, see column 5, line 58- column 6, line 17	1,4-10,13- 18,49,52-54, 61	X	US, A, 4,357,142, published 2 November 1982, Schall, Jr. et al, see column 6, lines 37-64	19,34
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>*</sup> Special categories of cited documents: <sup>15</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>																				
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">           Date of the Actual Completion of the International Search <sup>8</sup>            07 March 1986         </td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">           Date of Mailing of this International Search Report <sup>9</sup>            12 MAR 1986         </td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">           International Searching Authority <sup>1</sup>            ISA/US         </td> <td style="border-bottom: 1px solid black; padding: 5px;">           Signature of Authorized Officer <sup>10</sup>             Christine M. Nucker         </td> </tr> </table>			Date of the Actual Completion of the International Search <sup>8</sup> 07 March 1986	Date of Mailing of this International Search Report <sup>9</sup> 12 MAR 1986	International Searching Authority <sup>1</sup> ISA/US	Signature of Authorized Officer <sup>10</sup> Christine M. Nucker														
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